Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones

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We know very little about physiological constraints on the evolution of life-history traits in general, and, in particular, about physiological and molecular adjustments that accompany the evolution of variation in lifespan. Identifying mechanisms that underlie adaptive variation in lifespan should provide insight into the evolution of trade-offs between lifespan and other life-history traits. Telomeres, the DNA caps at the ends of linear chromosomes, usually shorten as animals age, but whether telomere rate of change is associated with lifespan is unknown. We measured telomere length in erythrocytes from five bird species with markedly different lifespans. Species with shorter lifespans lost more telomeric repeats with age than species with longer lifespans. A similar correlation is seen in mammals. Furthermore, telomeres did not shorten with age in Leach's storm-petrels, an extremely long-lived bird, but actually lengthened. This novel finding suggests that regulation of telomere length is associated not only with cellular replicative lifespan, but also with organismal lifespan, and that very long-lived organisms have escaped entirely any telomeric constraint on cellular replicative lifespan.

Keywords: bird; lifespan; mammal; senescence; survival; telomere

1. INTRODUCTION

Natural selection should oppose senescence, as long lifespans provide organisms with more reproductive opportunities. But, the senescent decline in survival and reproductive performance that individuals experience with advancing age is nearly universal in the life history of animals (Williams 1957). There is a wide range in maximum lifespans in different species. Species with long lifespans are particularly interesting because they may possess mechanisms that delay the onset of senescence and/or provide more effective defences against destructive ageing processes (Austad 2001). If we can identify physiological or molecular mechanisms that underlie the adaptive variation in lifespan, this may shed light on the evolution of trade-offs between lifespan and other life-history traits.

Birds live longer than mammals of similar body size, despite features of their biology that would be expected to decrease lifespan, such as high body temperatures and metabolic rates and blood glucose concentrations that exceed those found in mammals (Holmes & Austad 1995). Factors that affect maximum lifespan in birds, however, are not well known. Oxidative damage from free-radical production during oxidative metabolism (Harman 1956; Barja & Herrero 2000) and cell replicative senescence, a halt in the cell's ability to proliferate caused by the shortening of telomeres (West *et al.* 1989; Goldstein 1990; Vaziri *et al.* 1993) have been suggested as causal agents of ageing or age-related diseases. We report here that telomere rate of change (TROC) varies with maximum lifespan in birds and mammals, such that long-lived animals experience less-rapid telomere shortening than short-lived animals, and telomeres actually appear to lengthen with age in the longest-lived birds.

Telomeres, the termini of linear eukaryotic chromosomes, are involved in stabilizing chromosomal-end integrity (Prowse & Greider 1995), inhibiting the aberrant fusions and rearrangements that occur on broken chromosomes (McClintock 1941) and aiding the completion of duplication (Watson 1972). During each cell cycle, telomeric repeats $(T_2AG_3)_n$ are lost because DNA polymerase is unable to replicate the $3'$ end of linear DNA completely (Watson 1972), leaving a G-strand overhang. In some tissues, such as germ cells and carcinomas, telomeric repeats are maintained by telomerase, a ribonucleoprotein capable of elongating telomeres *de novo* (Greider & Blackburn 1985). In the absence of adequate telomerase activity, however, telomeres shorten with each cell division (Vaziri *et al.* 1994; Venkatesan & Price 1998), and we have recently shown that telomeres shorten with age in erythrocytes from zebra finches (Haussmann & Vleck 2002). In the present study, we measured telomere length in known-aged individuals of five bird species of different lifespans to determine whether telomeres from the nucleated blood cells (mainly erythrocytes) of these birds shorten with age and how the rate of shortening varies with maximum lifespan.

2. METHODS

(a) *Species*

We determined the relationship between telomere length and age in cross-sectional samples from zebra finches (*Taeniopygia*

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Table 1. *Avian species studied, average body mass and predicted and observed lifespans.*

species	body mass (kg)	predicted maximum lifespan (years)[a]	maximum observed lifespan (years)[b]	lifespan (percentage of predicted)
zebra finch[c]	0.012	7.3	5	69
tree swallow[d]	0.021	8.1	11	135
Adélie penguin[e]	4.600	23.9	20	84
common tern[f]	0.120	11.5	26	226
Leach's storm-petrel[g]	0.045	9.5	36	380

[a] Predicted lifespan based on body mass from the equation lifespan = 17.6 (mass in kg)$^{0.20}$ (Lindstedt & Calder 1976). [b] Based on published values for each species living in the wild. [c] Zann (1996). [d] Robertson *et al.* (1992). [e] Williams (1995). [f] Nisbet (2002). [g] Huntington *et al.* (1996).

guttata), tree swallows (*Tachycineta bicolor*), Adélie penguins (*Pygoscelis adeliae*), common terns (*Sterna hirundo*) and Leach's storm-petrels (*Oceanodroma leucorhoa*) (table 1). Zebra finch samples were collected from a captive colony maintained at Iowa State University since 1996. Tree swallow blood samples were collected from field sites near Ithaca, NY, from a study population followed since 1985 (Winkler & Allen 1996). Adélie penguin samples were obtained from captive known-age birds at San Diego SeaWorld, CA. Common tern blood samples were collected on Bird Island, MA, from a study population followed since 1970 (Nisbet *et al.* 2002). Leach's storm-petrel samples were collected on Kent Island, New Brunswick, from a population studied since 1947 (Huntington *et al.* 1996).

The reported maximum lifespans in the wild for these species range from 5 to 36 years. These values are based on long-term field studies with banding records for 2000 to 90 000 individuals per species. While there may be some error in these maximum lifespans, we think our estimates are robust, and greater accuracy is unlikely to change our conclusions. Body-size differences between species explain variation in a number of life-history parameters including lifespan, as larger animals usually live longer than smaller ones (Holmes & Austad 1995). Zebra finches, tree swallows and Adélie penguins have maximum lifespans similar to those predicted for birds of their size, whereas common terns and Leach's storm-petrels live considerably longer than predicted (table 1).

(b) Telomere length determination

We determined telomere restriction fragment (TRF) length in erythrocyte DNA. Fresh whole blood (*ca.* 50 µl) was collected from the brachial or jugular vein and immediately diluted in 100 µl of ice-cold 2% ethylenediaminetetraacetic acid. DNA was extracted from isolated erythrocyte nuclei using a salt-extraction alcohol-precipitation method for samples from zebra finches, tree swallows, Adélie penguins and Leach's storm-petrels and one-fifth of the common tern samples. The DNA from the remainder of the common tern samples was extracted from isolated erythrocyte nuclei using agarose plugs (Biorad, Hercules, CA, USA). Analysis of covariance (ANCOVA) indicated that there was no significant effect of extraction method on the relationship between TRF lengths and age ($F_{2,41} = 1.45$, $p = 0.23$), so all common tern samples were included in subsequent analyses, regardless of extraction technique. Genomic DNA was digested for 16 h with *Hinf* I (50 U; New England Biolabs Inc., Beverly, MA, USA) at 37 °C, and DNA concentration was determined using fluorometry. Approximately 10 µg of digested DNA fragments from each individual were separated on a 0.6% non-denaturing agarose gel (3 V cm^{-1}) for 21 h. We ran three ^{32}P-labelled Lambda/*Hind* III size markers on each gel to test for uniformity of DNA migration rate across the gel, and one bird sample was run four times on each gel to determine the intra- and inter-gel coefficients of variation (all less than 1.5% for each species).

Gels were then dried and hybridized for 16 h at 37 °C with ^{32}P-labelled $(C_3TA_2)_4$ oligonucleotides in hybridization solution. We used a phosphor imager system (Molecular Dynamics) to visualize the TRFs and densitometry (IMAGEQUANT v. 1.2) to determine the position and strength of the radioactive signal in each of the lanes over the range of 3–30 kb (determined using a Lambda/*Hind* III size marker). Average labelled TRF length in each lane was calculated as the mean of the optical density using the formula: $L = \Sigma(OD_i L_i)/\Sigma(OD_i)$, where OD_i is the densitometry output at position i, and L_i is the length of the DNA (in base pairs; bp) at position i.

3. RESULTS

Mean TRF length in erythrocytes decreased with age in zebra finches, tree swallows, Adélie penguins and common terns (figure 1). Unexpectedly, TRF length did not decrease with age in Leach's storm-petrel erythrocytes, but rather increased (figure 1). Even if the values for juvenile Leach's storm-petrels are removed, the relationship between age and TRF length in adults remains significantly positive (slope = 34 ± 13 (s.e.) bp yr^{-1}, $F_{1,24} = 7.06$, $p < 0.01$, $r^2 = 0.23$). Lack of telomere shortening with age in Leach's storm-petrels suggests that protection or preservation of telomeric sequence length is associated with exceptional longevity in this bird. TROC (the slope of the regression line for telomere length versus age) is the extent of telomere length change per year (usually shortening), although in our cross-sectional study we cannot rule out age-related selection effects on telomere length, e.g. the oldest individuals may be that subset of an age cohort with the slowest TROC. If this is so, our values for TROC may underestimate the true mean TROC for the species. In the case of Leach's storm-petrels the lack of overlap between hatchlings and very old individuals does suggest some mechanism for telomere augmentation in this species.

TROC correlates directly with maximum reported lifespan for these species (figure 2), such that the long-lived species experience less telomere shortening per year than do the short-lived species. If we use normalized lifespan (lifespan as a percentage of that predicted based on body
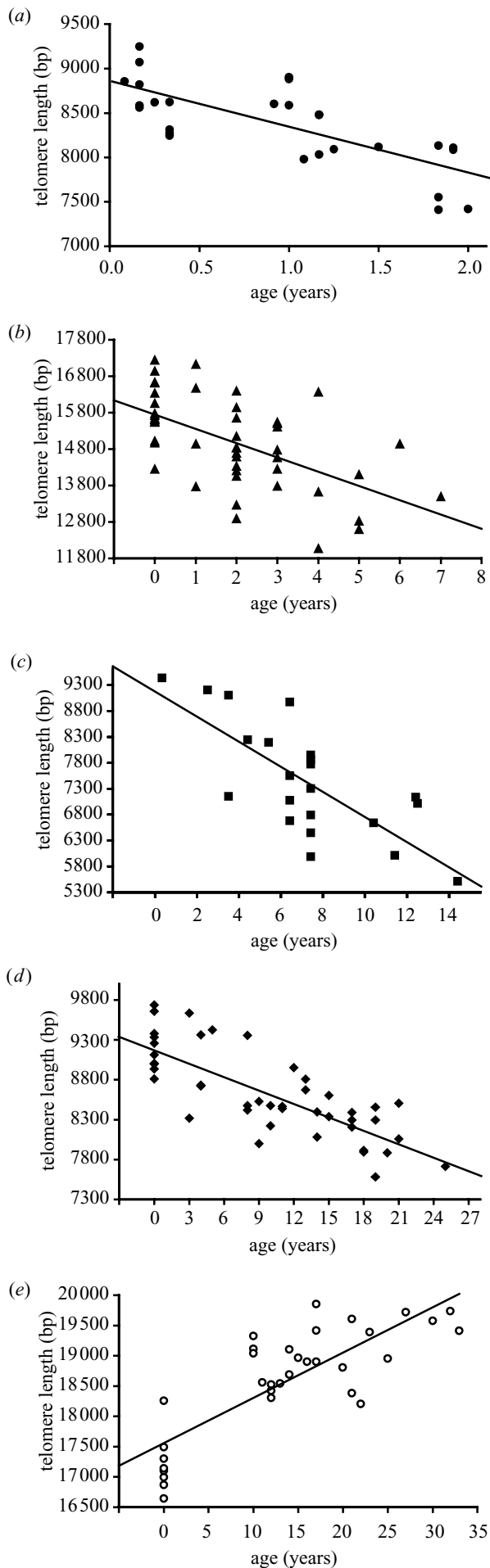
Figure 1. TRF length as a function of age in birds. The lines are the best-fit regressions through the data in (*a*) zebra finches (slope = -515 ± 95 (s.e.) bp yr^{-1}, $F_{1,26} = 29.9$, $p < 0.0001$, $r^2 = 0.54$); (*b*) tree swallows (slope = -391 ± 65 (s.e.) bp yr^{-1}, $F_{1,47} = 23.3$, $p < 0.0001$, $r^2 = 0.34$); (*c*) Adélie penguins (slope = -235 ± 48 (s.e.) bp yr^{-1}, $F_{1,21} = 23.9$, $p < 0.0001$, $r^2 = 0.55$); (*d*) common terns (slope = -57 ± 7 (s.e.) bp yr^{-1}, $F_{1,43} = 67.0$, $p < 0.0001$, $r^2 = 0.61$) and (*e*) Leach's storm-petrels (slope = $+75 \pm 10$ (s.e.) bp yr^{-1}, $F_{1,32} = 59.7$, $p < 0.0001$, $r^2 = 0.66$). No samples were obtained from Leach's storm-petrels between 1 and 9 years of age; young birds do not return to the breeding site for *ca.* 3–6 years after fledging.

mass), the relationship remains positive and significant ($F_{1,3} = 10.5$, $p = 0.05$, $r^2 = 0.78$). The data show no significant relationship between absolute length of telomeres early in life and lifespan ($p = 0.48$) (Vleck *et al.* 2003).

Because the between-species regression was based on only five taxa, we also assessed the significance of the relationship between maximum lifespan and TROC using a randomization test. Telomere length was randomly shuffled with respect to age (within species) for the 174 original birds; TROC was recalculated using these randomized data, and these values were correlated with species maximum lifespans. This procedure was repeated a million times. Only 215 randomizations had *F*-values greater than or equal to that in the observed data (*p*-value based on randomization = 0.000 215).

4. DISCUSSION

Many studies in a variety of tissues from mammals have shown a gradual decrease in TRF length with organismal age (Hastie *et al.* 1990; Coviello-McLaughlin & Prowse 1997; Frenck *et al.* 1998; Friedrich *et al.* 2000) and with doubling time in cell culture (Vaziri *et al.* 1994). This paper is the first, to our knowledge, to examine TROC as a correlate of species maximum lifespan. TROC is an important variable because it yields information on the number of telomere base pairs that are lost over time *in vivo*, rather than on how many base pairs are lost per cell division in culture. TROC explains 98% of the variation in maximum lifespan of the species studied, suggesting that this variable may be an important determinant of absolute lifespan, perhaps because it correlates with the time that the shortest telomere takes to reach a critical length (Hemann *et al.* 2001).

We found comparable TROC values for eight mammalian species (table 2). Only data from normal tissues (non-cancerous, HIV negative, non-germ cell) that spanned a significant portion of the species' lifespan were included. We excluded cell-culture data as these investigate the relationship between telomere length and population doublings, which does not reflect the rate of *in vivo* cellular division (Rohme 1981). Although there are well-known differences in TROC between different tissue types from a single organism, we included all the data here for completeness. TROC in mammals also shows a positive correlation with maximum lifespan (figure 3). If humans are excluded from the mammalian dataset to produce a range of maximum lifespans similar to that of the bird data,

Table 2. Telomere rate of change (TROC) and maximum lifespans in mammalian species taken from published literature (see § 4 for details).

species	tissue sampled	TROC (bp yr^{-1})	p-value[o]	n	age range sampled (years)	maximum lifespan[p] (years)
Mus spretus[a]	spleen	−600	0.0001	145	0–2	3.5
Mus spretus[a]	brain	−336	0.17	88	0–2	3.5
Canis familiaris[b]	mammary	−574	0.02	12	1–7	14
Canis familiaris[c]	leucocytes	−97[q]	0.57[q]	21	1–13	20
Ovis aries[d]	mammary	−590	0.01	18	1–6	20
Macaca nemestrina[e]	leucocytes	−440	—	12	2–9	26
Bos taurus[f]	leucocytes	−230	0.01	50	0–18	30
Macaca fascicularis[e]	leucocytes	−140	—	12	4–8	37
Macaca fascicularis[g]	leucocytes	−62.7	0.0001	55	0–34	37
Pan troglodytes[h]	leucocytes	−58[q]	—	1	followed for 14 years	53
Homo sapiens[i]	leucocytes	−97[q]	0.02[q]	5[r]	0–82	110
Homo sapiens[j]	leucocytes	−50	0.04	9	73–95	110
Homo sapiens[j]	skin	−78	0.006	7	73–91	110
Homo sapiens[j]	synovium	−25	0.5	8	73–95	110
Homo sapiens[k]	leucocytes	−33	0.001	50	13–90	110
Homo sapiens[l]	fibroblasts	−15	0.016	31	0–94	110
Homo sapiens[m]	stem cells	−68[q]	0.003[q]	11	0–59	110
Homo sapiens[n]	leucocytes	−55	—	2	followed for 8 years	110

[a] Coviello-McLaughlin & Prowse (1997). [b] Yazawa *et al.* (2001). [c] Nasir *et al.* (2001). [d] Shiels *et al.* (1999). [e] Shibata *et al.* (1999). [f] Miyashita *et al.* (2002). [g] Lee *et al.* (2002). [h] Feng *et al.* (1998). [i] Frenck *et al.* (1998). [j] Friedrich *et al.* (2000). [k] Hastie *et al.* (1990). [l] Allsopp *et al.* (1992). [m] Vaziri *et al.* (1994). [n] Feng *et al.* (1999). [o] Reported or calculated significance level for study (some papers did not provide a significance level). [p] Altman & Dittmer (1972). [q] Calculated from data in text or figure (some papers did not provide a significance level). [r] Calculated from mean values for five age cohorts.
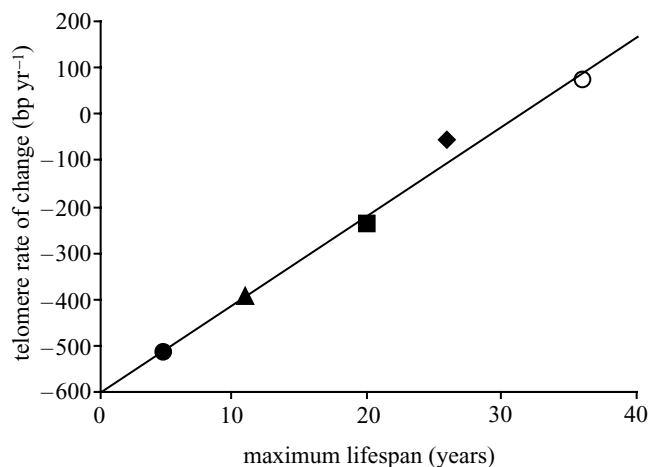

Figure 2. The TROC as a function of maximum observed lifespan in birds. Note that TROC is negative in most species, but positive in the Leach's storm-petrel. The line (TROC = −607 + 19.5 maximum lifespan) is the best-fit regression through the data (slope = 19.5 ± 1.2 (s.e.) bp yr^{-2}, $F_{1,3} = 262$, $p = 0.0005$, $r^2 = 0.99$). Black circle, zebra finch; triangle, tree swallow; square, Adélie penguin; diamond, common tern; open circle, Leach's storm-petrel.
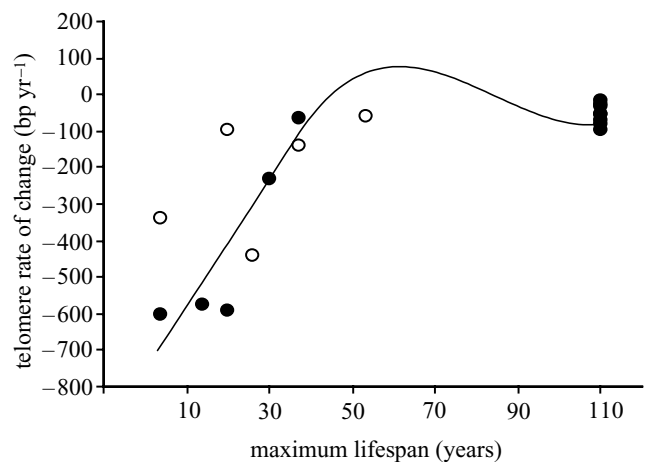

Figure 3. The TROC as a function of maximum observed lifespan in mammals. All available data are plotted, but only those data that had a significant TROC (closed symbols) have been fitted by using a lowess smoothing function and not those with non-significant TROCs (open symbols; see table 2 and § 4 for more details).

linear regression through the data produces a slope that does not differ from the slope in birds (slope = 17 ± 4.7 (s.e.) bp yr^{-2}, $F_{2,9} = 1.17$, $p = 0.15$). However, when humans are included, the relationship appears to be curvilinear in mammals. When we have data from the longest-lived birds, a similar curvilinear relationship may be revealed. Future comparative studies should also control for phylogeny.

The strong telomere length–lifespan relation in mammals is in accordance with the one we found for birds, despite the fact that the tissues sampled and methods employed differed markedly in the various mammalian studies. This concordance suggests that a fundamental link between telomere length and organismal lifespan may exist, perhaps resulting from the same mechanisms that link telomere shortening to cell replicative lifespan. We know that in many vertebrate species somatic cells demonstrate a limited proliferative capacity (Hayflick 1965), and

when this limit is reached these senescent cells contribute to age-related diseases (West *et al.* 1989; Goldstein 1990; Vaziri *et al.* 1993). Interestingly, a positive relationship between species lifespan and longevity of individual erythrocytes *in vivo* has been known for some time (Rohme 1981). If cellular turnover is slow in a given tissue, then TROC in that tissue will also be low because few cell divisions will occur per unit time.

Organismal and cellular lifespans should be positively correlated with other mechanisms that reduce the cumulative damage of ageing. Comparative studies have shown that rates of production of reactive oxygen species and levels of oxidative damage are lower in long-lived mammals than in short-lived mammals (Sohal *et al.* 1990; Ku & Sohal 1993; Barja & Herrero 2000). Birds appear to have lower rates of formation of reactive oxygen species (Ku & Sohal 1993; Barja *et al.* 1994) and are more resistant to oxidative damage (Ogburn *et al.* 1998) than mammals of comparable size but shorter lifespan. Based on cell-culture experiments, long-lived birds may be more resistant to damage by reactive oxygen species than short-lived birds (Ogburn *et al.* 1998, 2001). Our study suggests that long-lived animals may also be less susceptible than short-lived animals to cell replicative senescence caused by shortening telomeres. Reduced oxidative damage in long-lived species could also contribute to reduced telomere shortening, as the latter can be accelerated by oxidative damage (von Zglinicki *et al.* 2000).

Upregulation of telomerase activity could also delay telomere shortening, although telomerase activity is downregulated in most postnatal somatic tissue (Prowse & Greider 1995; Forsyth *et al.* 2002). Telomerase suppression may have been selected as a mechanism for reducing the frequency of cancer in somatic cells (Harley *et al.* 1994). In humans, telomerase activity is repressed in most somatic cell types including fibroblasts, embryonic kidney cells, lymphocytes and epithelial cells (Counter *et al.* 1992; Shay *et al.* 1993; Vaziri *et al.* 1993; Forsyth *et al.* 2002). While human haemopoietic stem cells possess some telomerase activity, telomeric DNA is nonetheless lost with age, suggesting that telomerase activity is inadequate to maintain telomere length in this cell type (Vaziri *et al.* 1994). Some mammalian tissues without telomerase activity are able to maintain telomere length through a mechanism termed the alternative lengthening of telomeres (ALT), but to date evidence for ALT activity has been found only in abnormal tissues (Henson *et al.* 2002). Variation in telomerase or ALT activity could explain species-level, individual and tissue-specific variation in telomere shortening. Long-lived storm-petrels might employ these mechanisms in somatic tissues such as haemopoietic stem cells, allowing telomere length to be maintained as they age. If so, it would be of considerable interest to learn how this species avoids the tumour susceptibility associated with these mechanisms.

If long-lived birds, such as storm-petrels, have evolved age-combating adaptations, such as active telomere elongation by upregulation of telomerase, freeing them from the constraint on cellular replicative lifespan normally imposed by shortening telomeres, then they may also provide insight into other fundamental questions in ageing biology. Here, we suggest that variation in TROC may be a molecular mechanism underlying the evolution of variation in species lifespans.

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